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Synthesis and two-dimensional ordering of asymmetrically polymer–brush–decorated cellulose nanocrystals

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Abstract

Rod-shaped cellulose nanocrystals (CNCs) are typically obtained by hydrolyzing cellulose nanofibers bearing an formyl group at their reducing end. In this study, we prepare CNCs bearing densely grafted polymer brushes of different molecular weights (asymmetrically polymer-brush-decorated CNCs; aPB-CNCs). aPB-CNCs exhibit good dispersion in organic solvents and form a monolayer at the air/water interface. The compression of this monolayer induces a phase transition of a long polymer brush at the reducing end and a change in the orientation of the CNC core due to repulsive interactions. A CNC is identified as a key intermediate, possessing dormant moieties for reversible addition–fragmentation polymerization and atom transfer radical polymerization at the reducing end and other surfaces, respectively (bifunctional dormant CNC). This functional CNC facilitates the polymerization of a wide range of monomers and enables the synthesis of aPB-CNCs with diverse chemical properties. The developed synthetic route not only provides a basis for exploring the ordered structure of nanoparticles but is also useful for modifying the reducing ends of CNCs.

Introduction

Colloidal crystals, which are formed through the ordered arrangement of dispersed colloidal particles, exhibit advantageous properties and functions that are not observed in individual particles or their disordered aggregates [1]. Understanding the self-assembly mechanisms of these dispersed colloidal particles is important for controlling and designing higher-order structures. One of these mechanisms

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is the phase transition from an isotropic to a colloidal crystal phase (for spherical particles; Kirkwood–Alder transition) [2] or nematic phase (for rod-like particles; Onsager transition) [3, 4] observed when the concentration of the colloidal dispersion exceeds a certain threshold. Such transitions can spontaneously occur in systems where particles exhibit only repulsive interactions, as the associated translational entropy gain compensates for the loss of configurational (for spherical particles) or orientational (for rodlike particles) entropy, i.e., the total entropy increases.

The Kirkwood-Alder transition is observed in spherical colloid dispersions with a steric repulsion potential derived from polymer brushes (semisoft colloidal crystals) [5, 6] as well as potentials derived from hard-sphere (hard colloidal) and electrostatic (soft colloidal) interactions. Among others, polymer brush systems offer the advantages of high dispersion stability and the ability to form colloidal crystals under high-salinity conditions and in nonaqueous solvents, i.e., in environments characterized by electrostatic interaction shielding. Depending on the graft density on a flat substrate or the chain length on a particle, solvent-swollen polymer brushes are categorized as semidilute or concentrated, affording different types of steric repulsion potentials due to the entropic interactions of polymer chains, i.e., mixing and conformational entropies [7]. The repulsion force-distance profiles of semidilute polymer brushes are well described via the scaling theory of de Gennes [8]. In contrast, a markedly steeper profile is observed for concentrated polymer brushes (CPBs), suggesting a greater compression resistance. Owing to such differences, polymer brush-modified spherical silica colloids in dispersion states can form different types of colloidal crystals with body-centered cubic, face-centered cubic, and random hexagonal close-packed structures, depending on the graft density and chain length of the polymer brushes [9, 10]. Given that CPB exhibits high steric repulsion and ultralow friction characteristics because of its high osmotic pressure, CPB-modified hybrid particles preserve their high dispersity and mobility, forming a self-assembled structure in the quasiequilibrium state, even in the case of concentrated dispersions.

In the Onsager transition, the orientational ordering of rodlike particles is caused by the excluded volume effect, affording lyotropic liquid crystals. Cellulose nanocrystals (CNCs), which are rod-shaped stiff nanoparticles obtained by hydrolyzing cellulose nanofibrils with sulfuric acid, are typical mesoscale aqueous systems; they form highly stable aqueous dispersions because of the electrostatic interactions among them due to their surface sulfate groups [11]. In dilute dispersions, CNCs form an isotropic phase characterized by randomly oriented long axes, whereas in condensed dispersions formed beyond a certain critical point, they form a chiral nematic phase because of the twisted geometry of CNCs and repulsive interactions between them. In the latter phase, layers with aligned CNCs are stacked, and their orientation gradually changes. Other examples of (chiral) nematic phases have been also reported for CNCs modified and stabilized with polymer brushes under high-salinity conditions and in nonaqueous solvents [12, 13], with the introduction of dispersion-stabilizing repulsive interactions being an important aspect. The aforementioned CPB modification is a sound methodology and has been successfully applied to rod-like inorganic nanoparticles [14, 15].

Considering the above, in this study, we tried to combine the two types of asymmetric entropic interactions, i.e., the orientational ordering of rod-like (nonspherical) particles and steric repulsion between position-selectively introduced polymer chains, focusing on CNCs as the core particles and attempting to asymmetrically introduce polymer brushes with different chain lengths. CNCs possess hemiacetal groups at one end (reducing end) and hydroxy groups on the other surfaces, which enables the position-selective introduction of grafted polymers to yield asymmetrically polymer-brushdecorated CNCs (aPB-CNCs) [16-18]. The synthesis of aPB-CNCs involved an intermediate with two position-selectively introduced types of dormant species for controlled radical polymerization via reductive amination and click reactions (copper-catalyzed azide-alkyne cycloaddition: CuAAC) [19] followed by sequential polymerization. Poly(methyl methacrylate) (PMMA) was used as a graft polymer because of its high affinity for a wide range of organic solvents and suitability for controlled radical polymerization. The PMMA brushes grafted onto CNCs were characterized via Fourier transform infrared (FT-IR) spectroscopy and size exlusion chromatography (SEC) to investigate the molecular characteristics of aPB-CNCs. The orientational ordering of aPB-CNCs was investigated at the air/water interface via surface pressure–area (π –A) isotherm measurements and transmission electron microscopy (TEM).

Experimental and methods

Materials

Whatman qualitative filter paper (grade 1) was obtained from Whatman plc (Maidstone, UK). A regenerated cellulose dialysis tubing (molecular weight cut offs of 8 kDa) was purchased from Repligen corp. (Massachusetts, USA). 2-Chloroethylamine hydrochloride (98%), 2-picolineborane (85%), 4-cvano-4-[[(dodecylthio)carbonothioyl]thio]pentanoic acid (R_{CDT}COOH) (97%), propargyl alcohol (98%), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl, 98%), 4-dimethylaminopyridine (DMAP; 98%), 2bromoisobutyryl bromide (BiBB; 98%), ethyl 2-bromoisobutyrate (EBiB; 98%), L-serine (99%), and bicinchoninic acid (BCA) disodium salt (98%) were purchased from Tokyo Chemical Industry. Co., Ltd. (Tokyo, Japan). NaN₃ (98%), sodium acetate (98.5%), acetic acid (99.7%), CuSO₄·5H₂O (99.5%), azobis(isobutyronitrile) (AIBN; 98%), and CuCl (99.9%) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sulfuric acid (64%), sodium ascorbate (98%), dehydrated pyridine (99.5%), and 4,4-dinonyl-2,2-bipyridine (dNBipy, 96%) were purchased from Fujifilm Wako Pure Chemical Corp. (Osaka, Japan). CuCl₂ (98%), and methyl methacrylate (MMA, 99%) were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Water for dispersing CNC and functional one was deionized using an Elix Essential UV3 instrument (Merck KGaA, Darmstadt, Germany). Water for the Langmuir-Blodgett (LB) subphase was further purified using a Milli-Q Advantage instrument (Merck). CuCl was purified by stirring in acetic acid overnight followed by filtration, washing with ethanol and diethyl ether, and drying under vacuum. Prior to use, MMA was purified using an alumina column to remove inhibitors. All other reagents and solvents were used as received without further purification.

Characterization

SEC analysis was performed using a Shodex GPC-101 system (Showa Denko K.K., Tokyo, Japan) with an RI-74S

differential refractometer (Shodex) and a column system comprising an LF-G guard column and two LF-404 (Shodex) columns. Tetrahydrofuran was used as an eluent at a flow rate of 0.3 mL/min at 40 °C. PMMA standards (Varian Inc., California, USA) were used for calibration. Transmission FT-IR spectra were recorded on a Nicolet 8700 FT-IR spectrometer (Thermo Fischer Scientific Inc., MA, USA) equipped with a liquid nitrogen-cooled mercury cadmium telluride detector using the KBr tablet method. The sample chamber was purged using a nitrogen generator (AT-10NP-CS; Airtech Japan, Ltd., Kanagawa, Japan). A calibration curve was prepared using an unmodified CNC-PMMA mixture with a known component ratio. Nuclear magnetic resonance (NMR) spectra were recorded on a JNM-ECS 400SS spectrometer (400 MHz; JEOL Ltd., Tokyo, Japan) using CDCl₃. The chemical shifts are reported as δ values using tetramethylsilane as an internal standard. TEM imaging was performed using a JEM-2100 microscope (JEOL, Ltd.) at an accelerating voltage of 200 kV.

The BCA assay was conducted to quantify the number of formyl groups at the reducing ends of CNCs [20–22]. A solution containing Na₂CO₃ (5.43 g), NaHCO₃ (2.42 g), and BCA disodium salt (0.194 g) in water (100 mL) was mixed with the CNC solution (1:1:2, v/v/v) and a solution containing CuSO₄·5H₂O (0.125 g) and L-serine (0.126 g) in water (100 mL), and kept at 75 °C for 30 min. The resulting solution was cooled to room temperature and its absorbance was measured at 560 nm using an ultraviolet–visible–near infrared spectrophotometer (UV-3600; Shimadzu Corporation, Kyoto, Japan). A calibration curve was constructed using glucose solutions of known concentrations.

 π -A isotherms were recorded using a LB system (364 mm × 75 mm; KSV Instruments, Ltd., Helsinki, Finland) equipped with a dipper and two barriers. The surface pressure was measured using a filter paper suspended from a balance. Sample dispersions in CHCl₃ (~0.1 w/v%) were spread on the surface of water filled in a trough at 25 °C. The barriers moved at a constant speed of 6 mm/min. The compressed film was deposited on an untreated carboncoated TEM grid using the horizontal deposition method, and the surface area per particle was determined via TEM observations of the monolayer transferred at 20 mN/m.

Preparation of CNC (1) via acid hydrolysis

The CNC suspension was prepared by hydrolyzing filter paper (Whatman; No. 1) with sulfuric acid as described in a previous study [23]. Briefly, the filter paper (20 g) was cut into small pieces and stirred in sulfuric acid (64%, 180 mL) at 50 °C for 35 min. The reaction mixture was quenched by adding deionized water (600 mL). The suspended solid was washed twice via centrifugation and dialyzed against deionized water until the pH of the dialysate became neutral to afford the CNC suspension. The average CNC thickness was determined as $\sim 10 \text{ nm}$ via atomic force microscopy (Fig. S1).

Propargylation of $R_{CDT}COOH$ (Synthesis of $R_{CDT}COOCH_2C \equiv CH$, 7)

 $R_{CDT}COOCH_2C \equiv CH$ was synthesized according to a previously reported method [24]. R_{CDT}COOH (0.507 g, 1.26 mmol), propargyl alcohol (0.250 g, 4.46 mmol), EDC·HCl (0.477 g, 2.49 mmol), and DMAP (0.0332 g, 2.49 mmol) were dissolved in dehydrated CH₂Cl₂ (60 mL), and the solution was stirred overnight at room temperature. The mixture was washed with deionized water (twice) and saturated aqueous NaCl (twice). The organic phase was dried over anhydrous Na₂SO₄ and concentrated to obtain the crude product, which was purified via flash column chromatography (eluent = hexane:ethyl acetate, 1:9 v/v) to obtain compound 7 (0.545 g, 1.24 mmol, 98% yield). ¹H NMR (400 MHz, CDCl₃, tetramethylsilane, δ): 0.88 (3H, t, $-CH_2 - CH_3$), 1.26 (16H, m, $-C_4H_8 - C_8H_{16} - CH_3$), 1.39 (2H, m, $-C_{3}H_{6} - CH_{2} - C_{9}H_{19}$), 1.69 (2H, quin, $-S - CH_2 - CH_2 - C_{10}H_{21}$), 1.88 (1H, s, $-C - CH_3$), 2.40, 2.55 (2H, m, $-CO - CH_2 - CH_2 - C -$), 2.50 (1H, t, $HC \equiv C -)$, 2.68 (2H, m, $-CO - CH_2 - CH_2 - C -)$, 3.33 (2H, t, $-S - CH_2 - C_{11}H_{23}$), 4.71 (2H, t, $HC \equiv C - CH_2 - O -).$

Synthesis of bifunctional dormant CNC

Synthesis of azide-modified CNCs (CNC-N₃, **2**): 2-azidoethylamine was synthesized according to a previously reported method [25, 26]. 2-Chloroethylamine hydrochloride (3.00 g, 25.9 mmol) and NaN₃ (3.36 g, 51.7 mmol) were dissolved in deionized water (30 mL), and the solution was stirred overnight at 80 °C to yield a stock solution of 2-azidoethylamine. The pH of the CNC dispersion (5.4 wt%, 55 g) was adjusted to 3 using a buffer comprising 0.1 M aqueous acetic acid (246 mL) and 0.1 M aqueous sodium acetate (54 mL) and stirred for three days at 70 °C. The resulting dispersion was supplemented with the 2-azidoethylamine solution (0.423 g, 3.46 mmol) and 2-picolineborane (0.738 g, 6.90 mmol) three times at 24 h intervals upon stirring. After the reaction was completed, the product was purified via centrifugation and dialyzed against deionized water for 7 d. The mixture was supplemented with toluene/N-methylpyrrolidone (NMP) and distilled to remove water to yield a dispersion of 2 in NMP (2.1 wt%, 48.1 g; 34% yield).

Synthesis of CNCs R_{CDT} -functionalized at the reducing end (3): A dispersion of 2 in NMP (2.1 wt%, 32.7 g) was supplemented with 7 (0.352 g, 0.724 mmol) and sodium ascorbate (0.790 g, 3.99 mmol). The solution was purged



Scheme 1 Synthesis of asymmetrically polymer brush-decorated cellulose nanocrystals (aPB-CNCs). a $H_2N(CH_2)_2N_3$, 2-picoline-borane, H_2O , pH 4, 70 °C, 3 d; (b) $R_{CDT}COOCH_2C \equiv CH$ (7), CuSO₄, ascorbic acid, NMP, room temperature, overnight; (c) BiBB, pyridine, NMP, DMAP, room temperature, overnight; (d) AIBN, $R_{CDT}COOH$, MMA, NMP, 60 °C, 18 h; (e) EBiB, MMA, CuCl, CuCl₂, dNBipy,

40 °C, 2 h. $R_{CDT}COOH = 4$ -cyano-4-[[(dodecylthio)carbonothioyl] thio]pentanoic acid, NMP = *N*-methylpyrrolidone, BiBB 2-bromoisobutyryl bromide, DMAP 4-dimethylaminopyridine, AIBN azobis(isobutyronitrile), EBiB ethyl 2-bromoisobutyrate, MMA methyl methacrylate, dNBipy 4,4-dinonyl-2,2-bipyridine

with Ar, supplemented with $CuSO_4.5H_2O$ (0.0996 g, 0.400 mmol) under Ar, and stirred at room temperature overnight. The mixture was poured into ethyl acetate. Subsequently, the precipitate was collected via centrifugation, suspended in NMP, and dialyzed against deionized water for 6 d. The mixture in the dialysate was supplemented with toluene/NMP and distilled to remove water and afford **3** (1.8 wt%, 39.4 g; yield ~100%).

Synthesis of bifunctional dormant CNCs (4): A dispersion of **3** in dehydrated NMP (1.8 wt%, 22 g) was supplemented with BiBB (1.90 g, 8.26 mmol), pyridine (1.31 g, 16.5 mmol), and DMAP (0.0673 g, 0.551 mmol) and stirred at room temperature overnight under Ar. The suspended solid was washed with water via centrifugation (15000 g, 10 min, twice). Subsequently, the precipitate was redispersed in NMP and poured into ethyl acetate, followed by collection via centrifugation (20000 g, 30 min). This procedure was repeated twice, and the suspended solid was redispersed in NMP to obtain a dispersion of **4** in NMP (60% yield). Elemental analysis: H 5.73%, C 42.3%, N 0.54%, Br 9.03%, S 0.51%, O 41.89%.

Reversible addition fragmentation chain transfer (RAFT) polymerization:

AIBN (1.1 mg, 0.007 mmol), $R_{CDT}COOH$ (13 mg, 0.032 mmol), and MMA (8.00 g, 79.9 mmol) were added to a dispersion of **4** in NMP (1.4 wt%, 8.0 g). The solution was purged with Ar for 10 min and then stirred at 60 °C for 18 h. After polymerization, the reaction mixture was centrifuged with acetone (20000 g, 30 min, six times) to remove the free polymer. The collected **5** was redispersed in NMP (44% yield). The SEC analysis of the free polymer yielded a number-average molecular weight (M_n) = 1.1×10^5 g·mol⁻¹ and a dispersion D = 1.3.

Atom transfer radical polymerization (ATRP): EBiB (0.047 g, 0.24 mmol), MMA (4.00 g, 39.9 mmol), and dNBipy (0.196 g, 0.48 mmol) were added to the dispersion of **5** in NMP (0.9 wt%, 4.0 g). The solution was purged with Ar for 10 min, supplemented with CuCl (0.032 g, mmol) and CuCl₂ (0.011 g, 0.08 mmol) under Ar, and stirred at 40 °C for 2 h. After polymerization, the mixture was centrifuged (acetone, 40,000 g, 30 min, five times) to remove the free polymer; the resulting polymer–brush–decorated CNC (**6**) was collected to obtain the corresponding dispersion in *N*,*N*-dimethylformamide (99% yield). The SEC analysis of the free polymer yielded $M_n = 4.2 \times 10^3 \text{ g} \cdot \text{mol}^{-1}$, D = 1.2.

Results and discussion

The synthesis of aPB-CNC (Scheme 1) involved sequential grafting based on two types of controlled radical polymerizations (RAFT polymerization and ATRP) capable of accommodating a wide range of polymer species and molecular weights. Dormant groups for RAFT polymerization and ATRP were introduced at the reducing end and the other surfaces of CNCs, respectively, to synthesize a bifunctional dormant CNC for the following two reasons: (i) RAFT polymerization should be performed prior to ATRP because the RAFT group may be activated during ATRP, and (ii) the reducing end should be modified before the surface hydroxy groups because of the reactivity and steric hindrance of the hemiacetal group. An azido group was introduced by reacting the reducing end with 2-azidoethylamine to subsequently and efficiently append a R_{CDT} group via the CuAAC click reaction [19] under mild



Fig. 1 Infrared spectra of 1-4

conditions. Owing to its explosive characteristics (C/N < 1), 2-azidoethylamine was synthesized in water and used without purification or isolation. The FT-IR spectrum of 2 exhibited a peak at 2125 cm^{-1} ascribed to the azido group (Fig. 1), confirming the introduction of the azido group to 2. The BCA assay [20-22] revealed that the amount of residual hemiacetal groups decreased from 23 to 9 µmol CHO/g after reductive amination, i.e., approximately 60% of the hemiacetal groups at the reducing end was aminated. This nonquantitative yield may be due to the detrimental effect of the hemiacetal group causing steric hindrance and mutarotation on the coupling probability [23, 27, 28]. Even in this case, the density of the azido groups at the reducing end was estimated to be 1.9 groups/nm². If most of azido groups could be converted to the dormant group for the RAFT polymerization, the achieved density was sufficiently high to produce CPB, as compared not only with the CPB threshold (>0.1 chains/nm²) but also with the following values; the theoretical maximum of PMMA-graft density (equivalent to the bulk state) was calculated to be 1.8 chains/nm² using the cross-sectional area of PMMA (0.56 nm²/chain) (see Supplementary Information), and experimentally, the graft density has not exceeded 0.8 chains/ nm^2 [7, 29].

 $R_{CDT}COOH$ was propargylated via acylation in the presence of EDC·HCl to yield 7. The corresponding ¹H NMR spectrum exhibited the peaks of the methylene group adjacent to the alkyne derived from propargyl alcohol and the terminal methyl of the dodecane group of the R_{CDT} moiety (Fig. S2). The integrated intensity ratio of these peaks indicated the successful synthesis and purification of 7. The subsequent coupling (CuAAC) reaction between 7 and 2 under mild conditions (room temperature) afforded 3. The FT-IR spectrum of 3 (Fig. 1) did not exhibit any azide peaks, confirming the almost complete consumption of the azido groups and successful introduction of the RAFT dormant group at the



Fig. 2 Infrared spectra of 4-6

reducing end of 2. Therefore, the density of the R_{CDT} groups introduced at the reducing end was assumed to equal that of the azido groups (1.9 groups/nm²). Next, a bromoisobutyryl (BiB) group was attached as an ATRP-dormant (ATRPinitiating) group to the residual hydroxy groups on the CNCside surface via acylation to yield 4. The FT-IR spectrum of 4 showed a peak for the BiB carbonyl group at 1750 cm⁻ (Fig. 1), suggesting its successful synthesis. The DS value of the BiB group in all cellulose chains of CNC ($DS_{BiB all}$) was estimated at 0.22 via elemental analysis (bromine content: 9.0%), where DS represents the average number of hydroxy groups replaced with substituents per anhydroglucose unit (AGU). Assuming that all BiB groups were located on the CNC surface, the surface density of BiB was calculated as 1.3 groups/nm² (DS_{BiB} for the surface chain ($DS_{BiB,surface}$): 1.0). Because the cellulosic chains at the CNC surface expose one C6, one C2, and one C3 hydroxy groups to the outer space for every two consecutive AGUs, the DS of the surface AGUs does not exceed 1.5 [30]. Therefore, the $DS_{BiB,surface}$ value obtained in this study suggests that two thirds of the accessible hydroxy groups on the CNC surface was modified through acylation with the BiB group. The densities of the RAFT and ATRP dormant groups considerably exceeded the graft density of typical CPBs (>0.1 chains/nm²) [7], which enabled the position-selective synthesis of the desired functional CNC, possessing two types of dormant groups (4) sufficiently dense to produce CPBs on the CNC surface.

Thus, we attempted to synthesize aPB-CNCs with polymer brushes of different molecular weights and position selectivity using **4** as bifunctional dormant CNCs for sequential surface-initiating graft polymerization via RAFT polymerization and ATRP. Figure 2 shows the FT-IR spectra of **5** and **6**. Compared with that of **4**, the spectra of **5** and **6** showed more intense carbonyl peaks, which indicated the successful grafting of PMMA and enabled the Table 1Molecularcharacteristics of poly(methylmethacrylate)by reversible additionfragmentation chain transfer(RAFT)polymerization andatom transfer radicalpolymerization (ATRP)

	$M_{\rm n}{}^{\rm a}$	D^{a}	w _{PMMA} /w _{CNC} ^b	$\sigma (\text{chains/nm}^2)^c$	σ^{*^d}
RAFT polymerization	1.1×10^{5}	1.3	0.099	0.13	0.073
ATRP	4.2×10^{3}	1.2	0.48	0.27	0.15

The $w_{\text{PMMA}}/w_{\text{CNC}}$, σ and σ^* values are determined on the basis of Eqs. (S1, S2, S5, S6, S9–S15) ^aDetermined by size exclusion chromatography

^bDetermined by infrared spectroscopy

^cGraft density

^dDimensionless graft density per cross-sectional area

quantification of the weight ratio of the grafted PMMA brushes to the CNC core (w_{PMMA}/w_{CNC}) (see Supplementary Information). The free PMMA simultaneously produced from the free dormant species, R_{CDT}COOH or EBiB, during each polymerization step was analyzed via SEC to estimate the molecular weight of the corresponding graft polymer on CNCs. Table 1 summarizes the molecular characteristics of the polymer brushes. The M_n and D values of the simultaneously produced free polymers were assumed to equal those of the graft polymers. Notably, the first step (RAFT polymerization) yielded a polymer with a larger molecular weight than the second step (ATRP). Small D values were observed in both cases, suggesting a well-controlled polymerization. The graft density (σ) and dimensionless graft density per cross-sectional area (σ^*) were calculated from the $M_{\rm n}$, $w_{\rm PMMA}/w_{\rm CNC}$, and CNC size (width: 10 nm (Fig. S1), length: 150 nm (Fig. S3)) (see Supplementary Information) [7]. The σ^* values (>0.07) at the reducing end and other surfaces of 6 indicated that the RAFT polymerization and ATRP resulted in the relatively dense grafting of PMMA brushes. The above results indicate that CNC was efficiently and position-selectively grafted with well-defined polymer brushes of different molecular weights via sequential controlled radical polymerization (RAFT polymerization \rightarrow ATRP) to form aPB-CNCs.

We expected that PMMA decorated aPB-CNC is a good nanoparticle for the study of the structure change characteristic to a rod-like system in two-dimensional monolayer at the air/water interface, because PMMA chains have the good ability to form a Langmuir monolayer at the air/ water interface without any dewetting and aggregation, and aPB-CNC exhibited high dispersity in a common spreading solvent, CHCl₃, for a Langmuir monolayer. To clarify the structure of aPB-CNC layers formed at the air/water interface, the CHCl₃ dispersion of aPB-CNC was spread on a water subphase, and after evaporation of CHCl₃, π -A isotherms were measured, and then, the formed layers transferred onto a grid were observed by TEM. The TEM observation revealed that most of aPB-CNC did not overlap, indicating successful formation of stable monolayers of aPB-CNCs at the air/water interface (Fig. 4, S3; the detailed discussion will be given below).



Fig. 3 Surface pressure–area $(\pi$ –A) isotherms of the Langmuir monolayer of aPB-CNCs during compression (black curve) and the related order parameter (n = 4, red dots)

The black curve in Fig. 3 shows the π -A isotherm of the Langmuir monolayer of aPB-CNC during compression. The area was corrected using the number density of aPB-CNC determined from the TEM image of the monolayer transferred at 20 mN/m (Fig. S3). The aPB-CNC isotherm could be roughly divided into three regions characterized by a (i) surface pressure of almost zero, (ii) rapid increase in surface pressure, and (iii) gradual increase in surface pressure upon compression. A similar π -A isotherm was observed with polyaniline-functionalized multiwalled carbon nanotube monolayer [31]. Region (i) corresponds to a gas state with isolated individual aPB-CNCs not forming any dense or compact monolayer (Fig. S5a), whereas regions (ii) and (iii) correspond to liquid phases where aPB-CNCs interacted with each other to form a monolayer [31-33]. Region (ii) displayed a shoulder at a lower surface pressure. A similar shoulder appeared during the compression of a monolayer of free linear PMMA at the air/water interface [34] and was attributed to a phase transition from one liquid-expanded state to another (LE-L'E transition) [35]. In addition to a surrounded shell layer of a short CPB, the examined aPB-CNC had a longer brush tail with a possibly coil-like conformation in the outer region (Fig. S6). Therefore, the



Fig. 4 Transmission electron microscopy images of the aPB-CNC monolayer transferred from the air/water interface at (a) 2, (b) 10, (c) 20, (d) 30, (e) 40, and (f) 50 mN/m. Arrows indicate the orientational direction determined from the order parameter

shoulder in region (ii) was ascribed to the LE (Fig. S5b) –L'E (Fig. S5c) transition of the long PMMA brush tail. A further increase in surface pressure upon compression in regions (ii) and (iii) suggested an intensification of the interaction among the rod-shaped particles, which could presumably behave as symmetric rods with polymer brush shells because of the shrinkage of the long-brush tail. This behavior suggests a concentration-dependent transition of orientation [31, 32].

The monolayer of aPB-CNCs at different stages of the compression process was transferred to a TEM grid and was imaged (Fig. 4). In the acquired images, the dark rods were assigned to the CNC cores, and their density increased upon compression, which resulted in a decrease in the occupied area. The dispersity of aPB-CNCs was high at low surface pressures (Fig. 4a, S5a), indicating that attractive interactions, such as capillary and van der Waals forces, were not as strong as those between micro- or nanoparticles at the air/ water interface [36, 37]. Therefore, we concluded that the structure of the aPB-CNC monolayer can be controlled by adjusting the surface pressure. To provide more details, the orientation distribution and order parameters of aPB-CNCs were evaluated via TEM imaging obtained at different surface pressures. The order parameter for materials aligned in the two dimension (S_{2D}) was calculated as

$$S_{2\mathrm{D}} = \frac{1}{N_{\mathrm{rod}}} \sum_{i=1}^{N_{\mathrm{rod}}} \cos 2\theta_i,\tag{1}$$

where N_{rod} and θ are the number and orientation angle of aPB-CNCs, respectively. In each TEM image, the



Fig. 5 Orientational distribution for the aPB-CNC monolayer transferred from the air/water interface. The orientational direction was set to 0°

orientation angle $\theta = 0^{\circ}$ was set in such a way to maximize S_{2D} ; the orientation distributions and S_{2D} values were determined as the averages of analyzing four different TEM images. The horizontal deposition method was used to capture the native state of aPB-CNCs on the water surface rather than the vertical deposition method, in which case the rod-shaped particles may be oriented owing to the flow-induced drag force of the meniscus between the substrate and water surface [38]. The estimated orientation distribution and S_{2D} values are presented in Figs. 3, 5, respectively, suggesting an isotropic state in a lower-surface-pressure region including the LE–L'E transition region (π < 15 mN/m) (Fig. S5b, c). Such a finding indicates that the horizontal deposition procedure was efficient without promoting the

additional orientation of aPB-CNCs. Furthermore, the rotational torque on the rod-shaped particles induced by the velocity gradient on the liquid subphase during uniaxial compression was very small, thus not orienting aPB-CNCs at surface pressures lower than 15 mN/m. When the surface pressure exceeded 20 mN/m, S_{2D} increased, implying that the rotational torque uniaxial compression of the twodimensional monolaver increased to promote the orientation of the rod-shaped particles in a direction perpendicular to that of compression in the regions where the interparticle interactions become stronger (Fig. S5d) [32, 38, 39]. When the monolayer was compressed to reach region (iii), S_{2D} further increased and the rate of increase in surface pressure slowed down, which may explain the existence of a plateau in the isotherm. Such results suggest that the closer-packing state of the aPB-CNC monolayer with the relevant repulsive interaction promoted a structural transition to a more ordered state owing to the Onsager transition [3, 40-42]or the uniaxial compression (Fig. S5e).

Conclusions

A bifunctional dormant CNC was synthesized bearing different types of dormant groups for controlled radical polymerization with different position-selective activation mechanisms attached to the reducing end (RAFT polymerization) and the other surfaces (ATRP), demonstrating that it can be converted into CNC with polymer brushes of different molecular weights (aPB-CNCs) via sequential controlled radical polymerization. The π -A isotherm and TEM observations of the aPB-CNC monolayer at the air/ water interface suggested that at low surface pressures, aPB-CNCs were oriented randomly, whereas higher surface pressures induced a phase transition of the long polymer brush attached to the reducing end, followed by the orientation of the CNC core. Thus, we expect that aPB-CNCs can form higher-order structures driven by different interactions, including long chains anchored to the reducing end and the CNC core. Additionally, given the versatility of RAFT polymerization and ATRP and their compatibility with a wide range of monomers, the developed procedure can be used to prepare aPB-CNCs with position-selectively introduced chemically-different polymer brushes for constructing a variety of higher-order structures.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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